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Coupling between membrane tilt-difference and dilation: a new “ripple” instability and multiple crystalline inclusions phases

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Abstract. – A continuum Landau theory for the micro-elasticity of membranes is discussed, which incorporates a coupling between the bilayer thickness variation and the difference in the two monolayers’ tilts. This coupling stabilizes a new phase with a rippled micro-structure. Interactions among membrane inclusions combine a dilation-induced attraction and a tilt-difference-induced repulsion that yield 2D crystal phases, with possible coexistence of different lattice spacings for large couplings. Inclusions favoring crystals are those with either a long-convex or a short-concave hydrophobic core.

Lipid molecules in water spontaneously form fluid bilayers in which hydrocarbon tails are shielded from contact with water [1], [2]. In nature, lipid membranes constitute the walls surrounding living cells, and generally host a large number of (protein) inclusions [3]. Self-assembled surfactant membranes can form various phases, *e.g.*, lamellar (L_α), vesicular (L_4), or sponge (L_3) phases. At low temperatures, the molecules tilt relative to the membrane normal, forming the $L_{\beta'}$ phase which has an internal degree of freedom similar to that of the liquid crystalline smectic- C phase. The coupling between tilt and membrane curvature can produce a shape instability yielding the $P_{\beta'}$ or “ripple” phase [4], [5]. Recently, a new degree of freedom has been introduced by Seifert *et al.*: a *tilt-difference* between the two membrane monolayers. The coupling between tilt-difference and membrane curvature can produce instabilities yielding rippled phases, bilayer tubules and bicontinuous phases [6], very much like in the case of membranes containing nematogens or anisotropic inclusions [7], [8].

In this letter, we study the effects of the coupling between tilt-difference and membrane dilation in ordinary L_α membranes. We find a new type of “ripple” phase that has not

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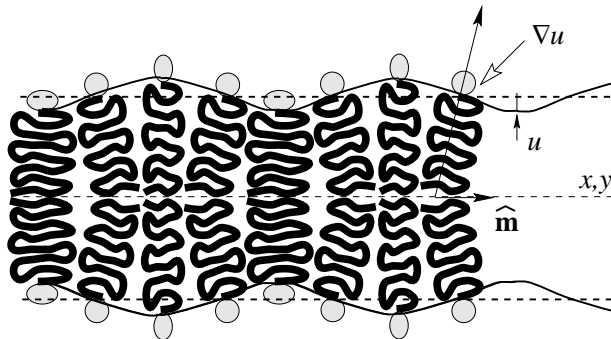


Fig. 1. – Lipid membrane subject to the ripple instability produced by the coupling $c \hat{\mathbf{m}} \cdot \nabla u$ between the dilation (u) and tilt-difference ($\hat{\mathbf{m}}$) modes.

yet been evidenced. The tilt-difference and dilation modes are generically excited by protein inclusions having a *convex* or a *concave* shape, or a hydrophobic core with a thickness different from that of the bilayer. We calculate and discuss the short-range interactions between such hosts, which form the most general up-down symmetric inclusions.

At lengths scales large compared with molecular dimensions, membranes are traditionally described by the shape $h(\mathbf{r})$ of their midsurface, with $\mathbf{r} = (x, y)$. Their elasticity is governed by the Helfrich curvature Hamiltonian $\frac{1}{2}(\kappa + \bar{\kappa})(\nabla^2 h)^2 - \frac{1}{2}\bar{\kappa}(\partial_i \partial_j h)(\partial_i \partial_j h)$ [9]. There is no $(\nabla h)^2$ surface tension term: membranes are self-assembled systems that optimize their area per molecule (or their coarse-grained area at larger scales.) We use here a different approach since we are interested in the *microscopic* elasticity of the bilayer [11]-[16]. To simplify, we only consider membranes perfectly symmetric with respect to their midsurface (a general theory treating the two monolayers independently will be presented elsewhere [10].) We develop a phenomenological Landau theory for the membrane dilation $u(\mathbf{r})$ (thickness variation), and the molecular tilt-difference $\hat{\mathbf{m}}(\mathbf{r})$. The latter is defined as half the sum of the projections onto the (x, y) plane of the unit vectors, oriented tail-to-head, parallel to the molecules in the two monolayers (fig. 1). The model free energy involves all quadratic terms and first order derivatives that satisfy rotational symmetry:

$$f = \frac{1}{2}B u^2 + \frac{1}{2}\lambda (\nabla u)^2 + c \hat{\mathbf{m}} \cdot \nabla u + \frac{1}{2}t' \hat{\mathbf{m}}^2 + \frac{1}{2}K'_1 (\nabla \cdot \hat{\mathbf{m}})^2 + \frac{1}{2}K'_2 (\nabla \times \hat{\mathbf{m}})^2. \quad (1)$$

Due to the tendency of the molecules to orient perpendicular to the chain-water interface, we expect $c > 0$. For biological membranes, the typical energy and length scales are given by $\kappa \simeq 25 k_B T$ (10^{-12} erg) and $\xi_0 \simeq 20 \text{ \AA}$ (monolayer thickness) [1]. This yields $B \approx \kappa/\xi_0^4 \simeq 6 \times 10^{14} \text{ erg cm}^{-4}$ which agrees with the experimental value of the area-stretching coefficient $B(2\xi_0)^2 \simeq 100 \text{ erg cm}^{-2}$ [1]. The dilation term $\frac{1}{2}B u^2$ has contributions from the water-chain(oil) surface tension and from the polymer-like stretching of the chains. The dilation-gradient term $\frac{1}{2}\lambda (\nabla u)^2$ therefore originates from the extra cost of *modulating* the stretching of the chains and increasing the water-chain area. Hence, we expect $\lambda \approx B\xi_0^2 \simeq 25 \text{ erg cm}^{-2}$, dimensionally, whether or not the (macroscopic, effective) tension of the membrane vanishes. On this point, our model disagrees with those of Dan *et al.* [18]. On the same basis, the other coefficients c , t' are expected to be $\approx \kappa/\xi_0^2$ and the K'_i 's are expected to be $\approx \kappa$. Neglecting higher-order gradient terms in (1) is in fact only justified when B is small, which is the case in the vicinity of a transition to a more ordered L_β or $L_{\beta'}$ phase. For a microscopic $\sqrt{\lambda/B}$, we expect nonetheless to get qualitatively correct results.

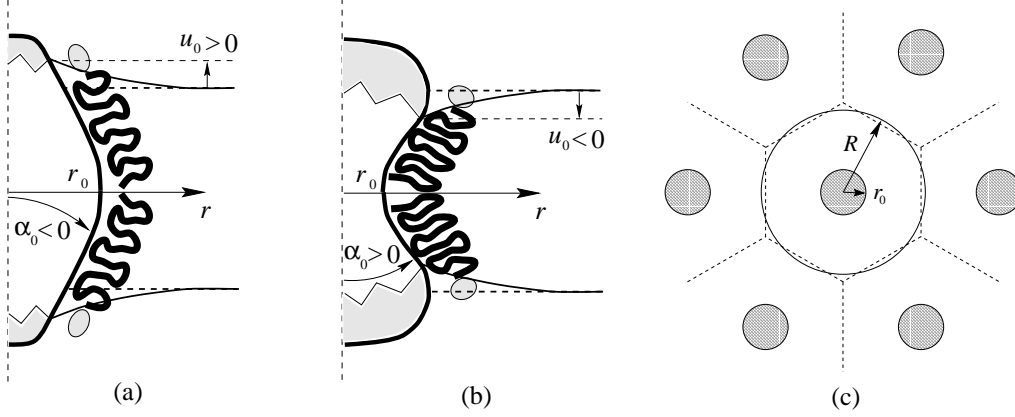


Fig. 2. – Protein inclusions with thickness mismatch u_0 and tilt-difference angle α_0 . Inclusions forming 2D crystals have either a long-convex (a) or a short-concave (b) hydrophobic core. (c) Wigner-Seitz cell approximated by a circle in an array of inclusions.

“Ripple” phase. – The linear stability of the flat membrane can be studied in the reciprocal space, where the free-energy density per mode associated with (1) can be diagonalized to

$$\frac{1}{2}B(k) \left| u_{\mathbf{k}} - i \frac{ck}{B(k)} \hat{m}_{\mathbf{k}}^{\parallel} \right|^2 + \frac{1}{2} \frac{N(k)}{B(k)} \left| \hat{m}_{\mathbf{k}}^{\parallel} \right|^2 + \frac{1}{2} t'(k) \left| \hat{m}_{\mathbf{k}}^{\perp} \right|^2, \quad (2)$$

where $\hat{m}_{\mathbf{k}}^{\parallel}$ and $\hat{m}_{\mathbf{k}}^{\perp}$ are the projections of the tilt-difference Fourier component $\hat{\mathbf{m}}_{\mathbf{k}}$ parallel and perpendicular to \mathbf{k} , respectively, and $B(k) = B + \lambda k^2 > 0$, $t'(k) = t' + K_2' k^2 > 0$, $N(k) = (t' + K_1' k^2)(B + \lambda k^2) - c^2 k^2$. The stability of the flat membrane is dictated by the sign of $N(k)$. When the latter is positive, the minimum energy corresponds to $u_{\mathbf{k}} = \hat{\mathbf{m}}_{\mathbf{k}} = 0$ and the flat membrane is stable. Defining the dilation-, the tilt-difference- and the coupling-characteristic lengths, as

$$\xi = \sqrt{\frac{\lambda}{B}}, \quad \xi' = \sqrt{\frac{K_1'}{t'}} \quad \text{and} \quad \ell = \frac{c}{2\sqrt{Bt'}}, \quad (3)$$

respectively, $N(k) < 0$ is equivalent to $\xi^2 \xi'^2 k^4 + (\xi^2 + \xi'^2 - 4\ell^2) k^2 + 1 < 0$. Therefore the instability occurs for $\ell > (\xi + \xi')/2$ at a nonzero wavevector $k_c = (\xi \xi')^{-1/2}$, the dilation and tilt-difference modulations being in quadrature (fig. 1).

Short-range interactions among inclusions. – Understanding the membrane-mediated interactions among inclusions has recently attracted much interest. Conical inclusions coupling to the local membrane curvature are subject to *long-range* elastic and Casimir (fluctuation) forces [19]–[22] that add up to the standard direct forces. On the other hand, *short-range* interactions arise from local structural perturbations: integral proteins have a central hydrophobic region spanning the hydrophobic core of the membrane and two polar extremities protruding outside. Any thickness mismatch between the protein and the bilayer hydrophobic regions results in a thickness perturbation that yields membrane-mediated interactions [12]–[16].

Here, we focus on the effects of the tilt-difference distortions that are naturally excited by inclusions with a convex or concave hydrophobic region. To simplify, we assume $\xi' \equiv \xi$ and hence $\ell < \xi$ for the membrane’s stability, and we restrict attention to inclusions having

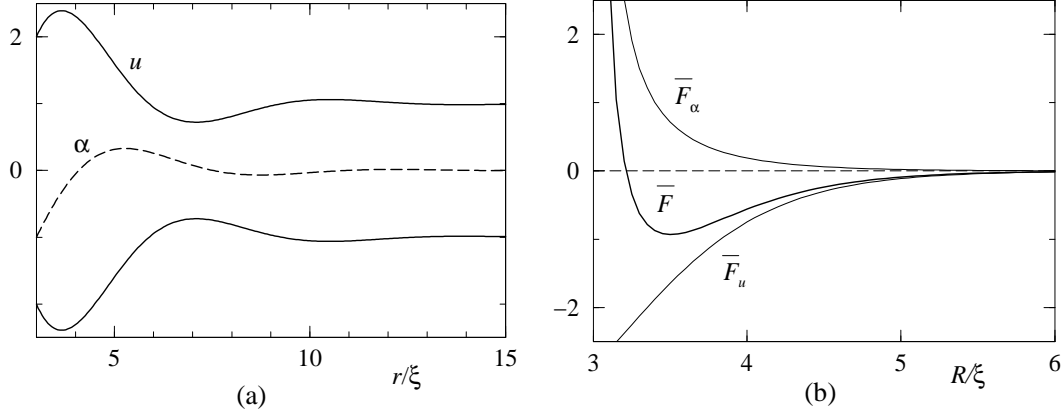


Fig. 3. – (a) Sketch of the membrane structure around an isolated inclusion with boundary dilation (u) and tilt-difference (α) normalized to 1 ($r_0/\xi = 3$, $x = -2$, $\phi = 0.75 \times \pi/2$). (b) Interaction energy \bar{F} in units of $10 k_B T$ vs. separation for zero dilation-tilt-difference coupling ($r_0/\xi = 3$, $x = 0.5$, $\phi = 0$).

revolution and up-down symmetry. We denote by u_0 their hydrophobic thickness mismatch and by α_0 their tilt-difference angle (fig. 2a,b). Their mean field interaction can be derived from the energy of the membrane equilibrium structure, which obeys the Euler-Lagrange equations associated with (1),

$$B u - \lambda \nabla^2 u = c \nabla \cdot \hat{\mathbf{m}}, \quad (4)$$

$$t' \hat{\mathbf{m}} - K'_1 \nabla (\nabla \cdot \hat{\mathbf{m}}) + K'_2 \nabla \times (\nabla \times \hat{\mathbf{m}}) = -c \nabla u. \quad (5)$$

To study the physics of a collection of inclusions we consider the Wigner-Seitz cell surrounding one inclusion with radius r_0 in a lattice (fig. 2c) and we approximate it by a circle of radius R [12], [14]. The problem therefore acquires revolution symmetry. In an hexagonal lattice, this amounts to neglecting high-order Fourier harmonics (6, 12, etc.), and in a gas to considering that the first neighbors effectively screen the other inclusions. Assuming radial symmetry, $u = u(r)$ and $\hat{\mathbf{m}} = \alpha(r) \hat{\mathbf{r}}$, the equilibrium solutions are given by the real part of

$$u(r) = \left[\mathcal{A}_1 K_0 \left(e^{i\phi} \frac{r}{\xi} \right) + \mathcal{A}_2 I_0 \left(e^{i\phi} \frac{r}{\xi} \right) \right] \times \sqrt{\frac{t'}{B}}, \quad (6)$$

$$\alpha(r) = \left[\mathcal{A}_1 K_1 \left(e^{i\phi} \frac{r}{\xi} \right) - \mathcal{A}_2 I_1 \left(e^{i\phi} \frac{r}{\xi} \right) \right] \times i, \quad (7)$$

where $i = \sqrt{-1}$, the I 's and the K 's are modified Bessel function, $\sin \phi = \ell/\xi$ ($\ell < \xi$), and \mathcal{A}_1 , \mathcal{A}_2 are two dimensionless complex constants that we determine from the boundary conditions:

$$u|_{r_0} = u_0, \quad \alpha|_{r_0} = \alpha_0, \quad u'(r)|_R = 0, \quad \alpha|_R = 0. \quad (8)$$

The distortion energy F stored in the region $r_0 < r < R$ [23] can be transformed by integration by parts into $F = \pi [\lambda r u u' + c r u \alpha + K'_1 (r \alpha' + \alpha) \alpha]_{r_0}^R$, which can be scaled to

$$\frac{F}{\pi B r_0 \xi u_0^2} = \bar{F} \left(x, \phi, \frac{r_0}{\xi}, \frac{R}{\xi} \right), \quad \text{with} \quad x = \frac{\alpha_0}{u_0 \sqrt{B/t'}}. \quad (9)$$

For typical integral proteins, we expect $r_0 \simeq 3 \xi$ with $\xi \simeq 20 \text{ \AA}$. Both the dilation and the tilt-difference distortion around an inclusions relax exponentially to zero on a distance $\simeq 100 \text{ \AA}$

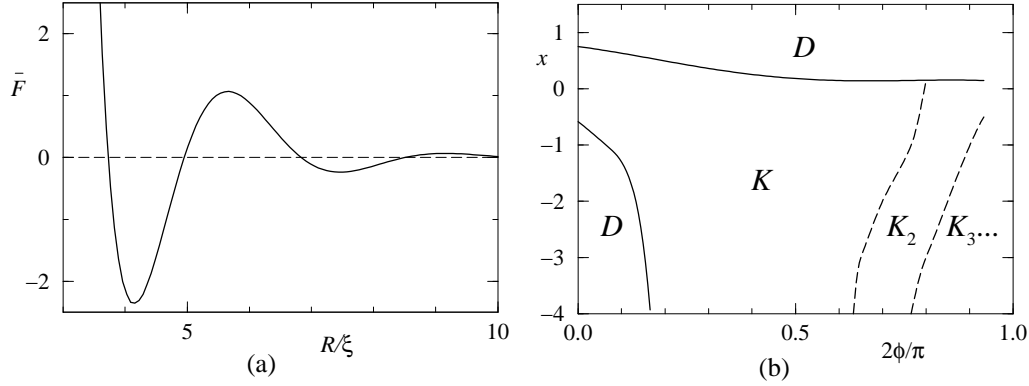


Fig. 4. – (a) Interaction energy \bar{F} in units of $10 k_B T$ vs. separation for nonzero dilation-tilt-difference coupling ($r_0/\xi = 3$, $x = -3$, $\phi = 0.75 \times \pi/2$). (b) Phase diagram for a membrane with $\xi \simeq 20 \text{ \AA}$ hosting inclusions with radius $r_0 \simeq 3\xi$. (D) disordered. (K) crystal. (K_n) region where n distinct stable or metastable crystalline phases are possible. For $x \simeq 0$, the crystal spacing is so small that the inclusions effectively aggregate.

($\simeq 5\xi$), as shown in fig. 3a. The coupling between dilation and tilt-difference generates oscillations and overshoots. For zero coupling, F can be splitted into a dilation-induced *attraction* F_u and a tilt-difference-induced *repulsion* F_α that combine to yield a minimum for small enough x (fig. 3b). With the values of the material constants previously discussed and typically $u_0 \simeq 0.2\xi$ ($\simeq 4 \text{ \AA}$), we expect $\alpha_0 \simeq x \times 10 \text{ deg}$, and $\pi B r_0 \xi u_0^2 \simeq 10 k_B T$, which sets the magnitude of the interaction energy, and in particular the depth of the well in fig. 3b. For large couplings, interferences between the membrane oscillations yield several energy minima (see the two wells of depths $\simeq 25 k_B T$ and $3 k_B T$ in fig. 4a).

Figure 4b shows a typical phase diagram for a collection of up-down symmetric inclusions. The criterion for a crystal phase is the existence of a well deeper than $k_B T$. Solid lines indicate first-order phase transitions; dashed lines separate regions where different metastable crystals with different lattice spacings can exist. Remarkably, crystal phases occur preferentially for $x < 0$, *i.e.*, for inclusions such as depicted in fig. 2a,b.

Discussion. – The instability produced by the coupling between tilt-difference and dilation defines a new “ripple” phase of membranes. The corresponding corrugation actually forms a *micro-structure*, since the undulation period compares with the bilayer thickness (except maybe in the vicinity of a L_β or $L_{\beta'}$ phase). The amplitude of the corrugation, governed by the bilayer dilation elasticity, will probably not exceed a few angstroms: these ripples will be difficult to detect by electron microscopy or STM techniques. The present phase cannot be mistaken with the $P_{\beta'}$ phase, which exhibits much larger (height) amplitudes $\simeq 45 \text{ \AA}$ [24].

Up-down symmetric membrane inclusions generally have a slightly convex or concave hydrophobic core of thickness different from that of the bilayer. Due to the strong effective attraction between hydrophobic parts, such inclusions will excite the coupled tilt-difference and dilatation modes, which in turn will mediate short-range interactions between them. The thickness mismatch creates an energetic dilation corona around the inclusions and yields an *attraction* between like inclusions: no extra distortion occurs when the coronas overlap since the boundary dilations match. The tilt-difference, however, yields a *repulsion* between like inclusions: going from α_0 to $-\alpha_0$, it develops a strong gradient when the coronas overlap. Inclusion producing no tilt-difference aggregate, while inclusions producing a nonzero tilt-difference either repel one another or form $2D$ crystals. The latter situation arises for small

tilt-differences, or when the dilation corona extends further than the tilt-difference corona ($\xi > \xi'$). In both cases, the attraction which dominates at “large” distances is overcome by the repulsion at short distances.

When the coupling between dilation and tilt-difference is large, the distortions in the coronas exhibit damped oscillations and the interparticle potential develops several minima. This implies the possible coexistence of different crystals of inclusions having different lattice spacings, and also the possibility of low density crystals (separation between inclusions’ boundaries $\simeq 180$ Å for the secondary minimum in fig. 4). The inclusions most likely to form 2D crystals are those with either a *long-convex* or a *short-concave* hydrophobic core, *i.e.*, those disfavored from the point of view of the $\hat{\mathbf{m}} \cdot \nabla u$ coupling. This is because the gradient of u being more costly, the dilation corona extends (favoring “long-range” attraction), while due to the effective shift in the tilt-difference’s quadratic potential, the dilation corona shrinks (making the repulsion occur only for smaller separations). Conversely, short-convex and long-concave inclusions have a dominant repulsion and should form disordered phases.

In replacing the Wigner-Seitz cell by a circle, we have neglected high-order Fourier harmonics. Taking them into account would probably change the detail of the interaction potential, but not its essential features. Real inclusions are not up-down symmetric: their average conical shape effectively adds a monotonic repulsion [10], which can be neglected provided the corresponding angle is small compared to the tilt-difference angle.

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